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Frequent Site-Specific Deletion of Coliphage λ Murine Sarcoma Virus Recombinants and Its Use in the Identification of a Retrovirus Integration Site

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Stocks of hybrid λ phages carrying the complete integrated provirus of either ml or HT1 Moloney murine sarcoma virus, as well as flanking host sequences, frequently contain significant numbers of phages carrying a specific deletion. This deletion arises from a recombination event between the terminally repeated sequences in the provirus that deletes the unique Moloney murine sarcoma virus sequences bracketed by the terminally repeated sequences. Physical mapping has shown that the deletion phage retains one complete copy of the terminally repeated sequence and the flanking mink host sequences. One such deletion, λHT1r$^+$, was used to characterize a mink genomic DNA sequence that contains an HT1 Moloney murine sarcoma virus integration site. This integration site sequence from normal mink cells was also cloned into phage λ. An analysis of the heteroduplexes between the integration site and the λHT1r$^+$ deletion indicated that no major rearrangement of host sequences occurred upon integration of the Moloney murine sarcoma provirus.

An obligatory intermediate in the life cycle of retroviruses is the integration of proviral DNA into the host genome (31, 36). The mechanism and specificity of this event are of considerable interest. By using restriction enzymes and hybridization, many investigators have established that retrovirus integration involves covalent linkage of proviral DNA to host DNA. There is evidence for both multiple and preferential sites of integration in hosts (2, 10, 14, 16, 18, 28). The portion of the viral DNA joined to cellular DNA is not random, but rather is a terminally repeated sequence (TRS) near or at the mature ends of the genome (14, 34). However, the methods used to establish these concepts do not allow us to examine the structure of integrated DNA at the nucleotide level. For this reason, we cloned in bacteriophage λ the integrated genomes of two related Moloney murine sarcoma viruses (MSV), ml MSV and HT1 MSV (34). The integrated forms of these viruses were isolated from transformed mink cells to overcome problems of related endogenous viruses in mouse cells. In this report we show how cloned proviral sequences provided us with tools to study in some detail one site of MSV integration in the mink genome. In particular, we describe a frequently encountered deletion that occurs specifically in the cloned fragments bearing integrated MSV genomes.

We have shown that integrated ml and HT1 MSV proviruses have directly repeated terminal sequences (34; G. F. Vande Woude, M. Oskarsson, W. McClements, L. Enquist, D. Blair, P. Fishinger, J. Maizel, and M. Sullivan, Cold Spring Harbor Symp. Quant. Biol., in press) which are apparently identical to the 600-base pair (bp) terminal repeats in unintegrated Moloney murine leukemia virus (M-MuLV) provirus (11). Such terminally redundant sequences have been reported in integrated and unintegrated avian proviruses (14, 24, 30). Restriction maps of the *Eco*RI fragments inserted into λml and λHT1 are shown in Fig. 1 (34; Vande Woude et al., in press). These maps show unique MSV sequences bounded by direct TRS and the entire MSV provirus flanked by mink host sequences. The TRS should serve as substrates for generalized recombination during replication of the hybrid phages λml and λHT1 in *Escherichia coli* (3, 22). If during growth of λMSV hybrids in *E. coli* the MSV TRS participate in either an intramolecular recombination event or an unequal crossing over between two phages, one resultant recombinant phage would lose all of its unique MSV sequences and retain one copy of the repeat and the flanking mink sequences. There was preliminary evidence for significant amounts of phage with this type of deletion in primary lysates of λHT1 and λml. Therefore, we tried to isolate and characterize these phage deletions, which could be of use in our study of

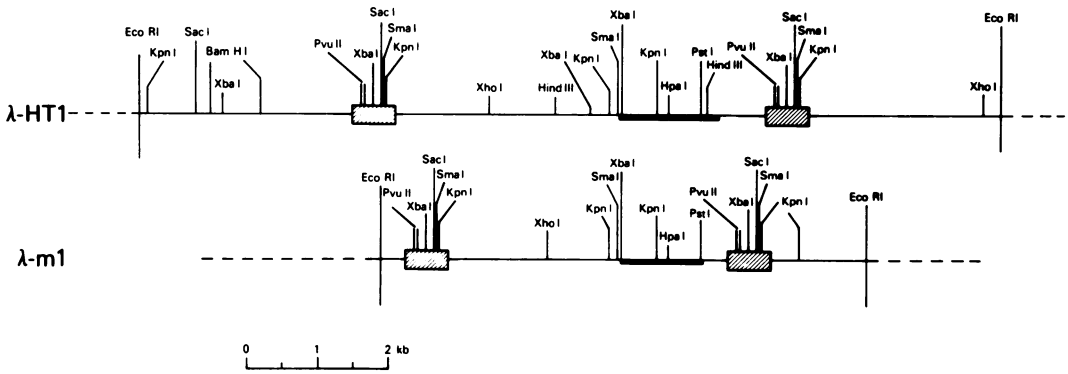

FIG. 1. *Physical maps of λHT1 and λm1. These maps show some of the restriction endonuclease sites in the inserted EcoRI DNA fragments of λHT1 and λm1 (34; Vande Woude et al., in press; McClements et al., manuscript in preparation). Additional PstI, BglII, and PvuII sites in the MSV and mink flanking sequences are not shown. The solid horizontal lines represent the insert DNA, the heavy lines represent the cellular mouse sequences known as "src," the cross-hatched rectangles are the terminal repeats, and the dashed lines indicate parts of the λgtWESB vector arms.*

MSV and the mechanism of retrovirus integration.

MATERIALS AND METHODS

Cells and viruses. The M-MuLV, MSV, and mink cell lines used here have been described previously (34).

DNA cloning procedures. The purification and identification of specific fragments from the mink genome by RPC-5 chromatography and preparative gel electrophoresis have been described elsewhere (32, 33). The λgtWES·λB vector system was used as detailed previously (17). The growth of recombinant DNA phages and subsequent isolation of phage DNA were performed under P2-EK2 containment conditions.

DNA analysis. Restriction endonuclease digestion of DNA with *Eco*RI (Boehringer Mannheim Corp.) or *Xba*I and *Sac*I (New England BioLabs) was done as recommended by the manufacturers. Analytical comparisons of digested DNA were performed by electrophoresis in agarose gels (32, 33) or on acrylamide gels. Blotting of DNA onto cellulose nitrate membranes was as described by Southern (27). ^{32}P-labeled M-MuLV complementary DNA was prepared by the endogenous reverse transcription reaction, as described previously (34). Heteroduplex formation and analysis have been described elsewhere (32).

Isolation of λ hybrids carrying deletions. (i) CsCl gradients. High-titer phage lysates prepared on *E. coli* DP50supF (17, 34) were clarified by low-speed centrifugation. A sample containing about 10^8 phage was mixed with a marker phage solution containing 10^6 PFU of each marker. This solution was mixed with enough solid CsCl to give a density of 1.5 g/ml and was centrifuged to equilibrium in an SW56 rotor (20°C, 35,000 rpm, 20 h). About 50 fractions were collected in tubes containing 0.5 ml of 0.05 M Tris (pH 7.4)–0.01 M $MgSO_4$–0.01% gelatin. Fractions were assayed for plaque-forming phage by standard methods.

(ii) EDTA treatment. Phage lysates were diluted at least 50-fold into 0.02 M EDTA–0.02 M Tris (pH 7.4) and heated at 41°C for 15 min (20). The solution was then made 0.05 M in $MgSO_4$ by adding an appropriate volume of 1 M $MgSO_4$. Phage surviving this treatment were isolated, and their EDTA resistance was confirmed. Virtually all isolates obtained in this way contained deletions.

RESULTS

Isolation of a novel deletion of λMSV phages. A specific deletion of λHT1 could be found in every lysate of the hybrid phage. We first noticed this in DNA preparations of λHT1 when total phage particles were purified by glycerol sedimentation (34). When cleaved with *Eco*RI, such DNA contained a 6.2-kilobase (kb) fragment in addition to the 12.3-kb fragment bearing the integrated HT1 MSV provirus. The 6.2-kb fragment could be detected even after several cycles of single plaque isolation. The size of the fragment was always 6.2 kb, but its abundance varied from lysate to lysate. The constant size of the 6.2-kb fragment in many independent lysates strongly indicated that a specific deletion was occurring within the inserted DNA.

Isolation of the phage carrying the small fragment was accomplished by two independent procedures. In one, deleted phages, which were less dense than parental phages, were separated by isopycnic centrifugation in CsCl. In the other, the lysates were treated with EDTA, which preferentially ruptured phages with normal-sized λ DNA (49.5 kb) but left intact those phages with smaller genomes (21). Both methods readily revealed the presence of a novel phage in λHT1 lysates. Figure 2 shows the density distribution of plaque-forming phages obtained by CsCl iso-

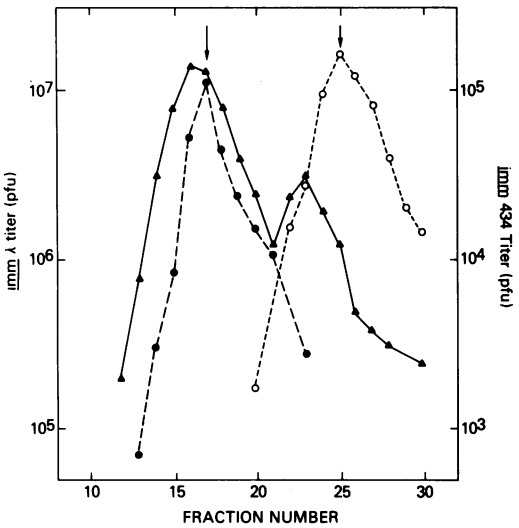

FIG. 2. *Density distribution of plaque-forming phage in a lysate of λHT1 by CsCl isopycnic centrifugation. About 10^8 phage from one 75-ml lysate prepared as described previously (24) were mixed with two heteroimmune marker phages and centrifuged to equilibrium as described in the text. The two marker phages were λ imm434 (48 kb) and λ b538 imm434 c Sam7 (39.8 kb). The marker phages could be distinguished from λHT1 phage by their immunity phenotypes; imm434 phage grow on YMC(λ) but not on YMC(434), whereas immλ phage (λHT1) grow on YMC(434) but not on YMC(λ). The two imm434 marker phages were distinguished as follows. λ imm434 produces turbid plaques, whereas λ b538 imm434 c Sam7 produces clear plaques. Symbols: ○ and ●, density profile of the imm434 phage (○, clear plaques; and ●, turbid plaques); ▲, immλ phage from the λHT1 lysate. The arrows indicate the peak positions for density calculations.*

pycnic centrifugation of a typical λHT1 lysate. Marker phages of known densities and DNA contents were included. A new peak of less dense phage, representing about 40% of the total plaque-forming particles, was observed. By comparison with marker phages, the genome size of this new phage was about 42 to 43 kb, or about 6 kb smaller than λHT1. Phage were purified from the light-density peak, and several single plaque isolates were analyzed. As is shown below, all isolates were identical and carried a 6.2-kb *Eco*RI fragment between the two normal λgtWES arms. These λHT1 recombination-deleted (λHT1r$^+$) phage were stable and could be propagated with no further changes. Phage with 6.2-kb inserts were also obtained as EDTA-resistant survivors in λHT1 lysates.

The same type of unique deletion could be detected in λm1 stocks under special circumstances. Evidence for a specific deletion was found in another recombinant phage, λm1ff, which was isolated at the same time as λm1 and carried in addition to the 7-kb fragment in λm1 a slightly larger *Eco*RI fragment from the mink genome. When a lysate of λm1ff was banded in CsCl, a small peak that was less dense than the bulk of the plaque-forming phages appeared (data not shown). Several isolates of this less dense peak were purified, and the DNA was isolated. These phage are called λm1r$^+$.

Analysis of λMSV deletions. DNAs from the parental and deletion phages were analyzed by restriction endonuclease *Eco*RI digestion. Results of fractionation of these digests by agarose gel electrophoresis are shown in Fig. 3. Figure 3a shows the ethidium bromide-stained gel; tracks 1 through 5 contained DNA from λHT1, λHT1r$^+$, λm1, λm1ff, and λm1r$^+$, respectively. The three largest bands in each track (at 36, 22, and 14 kb) are the λgtWESB arms joined by their sticky ends, the λgtWESB left arm, and the λgtWESB right arm, respectively. The 12.3-kb band in λHT1 (Fig. 3a, track 1) was missing from λHT1r$^+$ and was replaced by a 6.2-kb fragment. This 6.2-kb fragment was also detected in track 1, indicating that the amount of λHT1r$^+$ was significant in the λHT1 phage population. In the series of experiments with λm1 (Fig. 3a, tracks 3 through 5), a 7.0-kb fragment was observed in λm1 (track 3). This fragment, which contained the m1 MSV provirus, was also present in λm1ff (track 4), along with the additional, slightly larger insert. In λm1r$^+$ (track 5) the MSV-containing fragment was replaced by a new *Eco*RI insert of approximately 2 kb, whereas the larger, non-MSV insert remained unchanged, providing evidence for the specificity of this deletion event.

We next determined that the new, smaller DNA inserts in λHT1r$^+$ and λm1r$^+$ retained some proviral sequences. The gel was analyzed by the method of Southern (27), using ^{32}P-labeled M-MuLV complementary DNA as a probe. As expected, the probe hybridized to the MSV sequences of M-MuLV origin in the 12.3-kb fragment (Fig. 3b, track 1) and the 7-kb fragments (Fig. 3b, tracks 3 and 4). The probe also hybridized to the new 6.2-kb fragments (tracks 1 and 2) and the 2-kb fragment (track 5). No hybridization to the λgtWESB arms or to the ≅7-kb non-MSV insert in λm1ff or λm1r$^+$ was detected with this probe. Therefore, these new DNA fragments retained M-MuLV-specific sequences.

To determine which sequences were retained, we formed heteroduplexes between the *Eco*RI inserts of λHT1r$^+$ and its progenitor, λHT1 (Fig. 4). Measurement of 20 molecules gave the sizes of the two double-stranded segments as 3.46 ± 0.30 and 2.62 ± 0.27 kb and the size of the single-

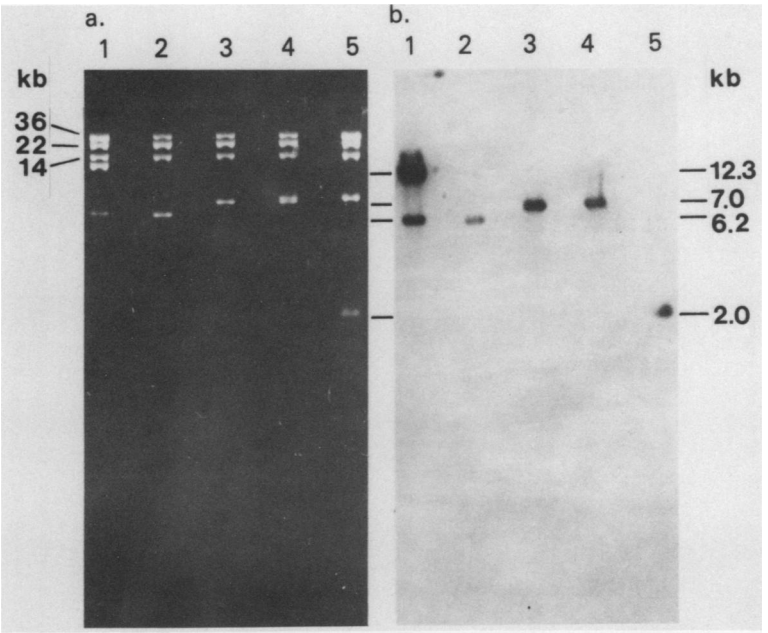

FIG. 3. *EcoRI analysis of parental and recombination-deleted phage DNA. DNAs from λHT1, λm1, and λm1ff and from deleted phages (λHT1r⁺, λm1r⁺) were prepared, digested with EcoRI, and fractionated by electrophoresis on a 0.7% agarose gel. Track 1, λHT1; track 2, λHT1r⁺; track 3, λm1; track 4, λm1ff; track 5, λm1r⁺. (a) Ethidium bromide-stained gel. (b) Autoradiogram of blotted gel in (a) hybridized with ^{32}P-labeled M-MuLV complementary DNA (specific activity ~2 × 10⁷ dpm/μg).*

stranded loop as 6.2 ± 0.29 kb. The size and position of the deletion loop in this heteroduplex support the idea that it is the unique MSV sequences that are deleted from λHT1r⁺ (Fig. 1), whereas the flanking mink sequences are retained, along with some sequences that hybridize to M-MuLV complementary DNA. Unlike a simple deletion loop that is fixed on the heteroduplex, the position of the deletion loop in these heteroduplexes shifts over a range of approximately 800 bp, a range greater than that expected from measurement error alone. Our interpretation is as follows. The presence on one DNA strand of one copy of the TRS (from λHT1r⁺) and on the other strand of two copies of the TRS (from λHT1) should form a loop that can branch migrate, allowing a 600-bp shift. Figures 4c and d show two forms of the branch migrating loop that could arise in such heteroduplexes. The heavy lines represent the TRS. Figure 4c shows the extreme case, in which one λHT1 TRS is completely duplexed with the single λHT1r⁺ TRS, whereas the other λHT1 TRS is entirely in the single-stranded loop. Figure 4d shows an intermediate case, in which parts of both λHT1 TRS are duplexed. The graphic representation of measured heteroduplexes in Fig. 5 illustrates this point. Each horizontal line represents the normalized length of the double-stranded segments of one duplex, and the ticks indicate the positions of the deletion loop. The range of these positions is 0.13 of the fractional length, which is equivalent to approximately 800 bp. Within the experimental error, these results are consistent with the branch migration expected in this type of heteroduplex and indicate that λHT1r⁺ contains one copy of the TRS.

The nature of the deletion in λHT1r⁺ was characterized further by restriction endonuclease mapping. The TRS of M-MuLV (11) and MSV (34; Vande Woude et al., in press) contain a pair of *Sac*I and *Xba*I sites about 118 bp apart (Fig. 1). Figure 6 shows an ethidium bromide-stained polyacrylamide gel of λHT1 and λHT1r⁺ digested with *Xba*I, *Sac*I, and *Xba*I plus *Sac*I. Figure 6 tracks 2 and 3 contained *Sac*I-digested λHT1 and λHT1r⁺, respectively; no small fragments were resolved. Tracks 4 and 6 contained *Xba*I-digested λHT1 and λHT1r⁺, respectively. The 450-bp fragment in λHT1 came from the region between the 3.4-kb *Xba*I site and the 3.9-kb *Xba*I site at the leukemia-sarcoma sequence joint (34; Vande Woude et al., in press) and was missing from λHT1r⁺. Tracks 7 and 8 contained *Sac*I-*Xba*I double digests of λHT1 and λHT1r⁺, respectively. The three λHT1 fragments resolved in track 7 were (i) a 450-bp *Xba*I-*Xba*I

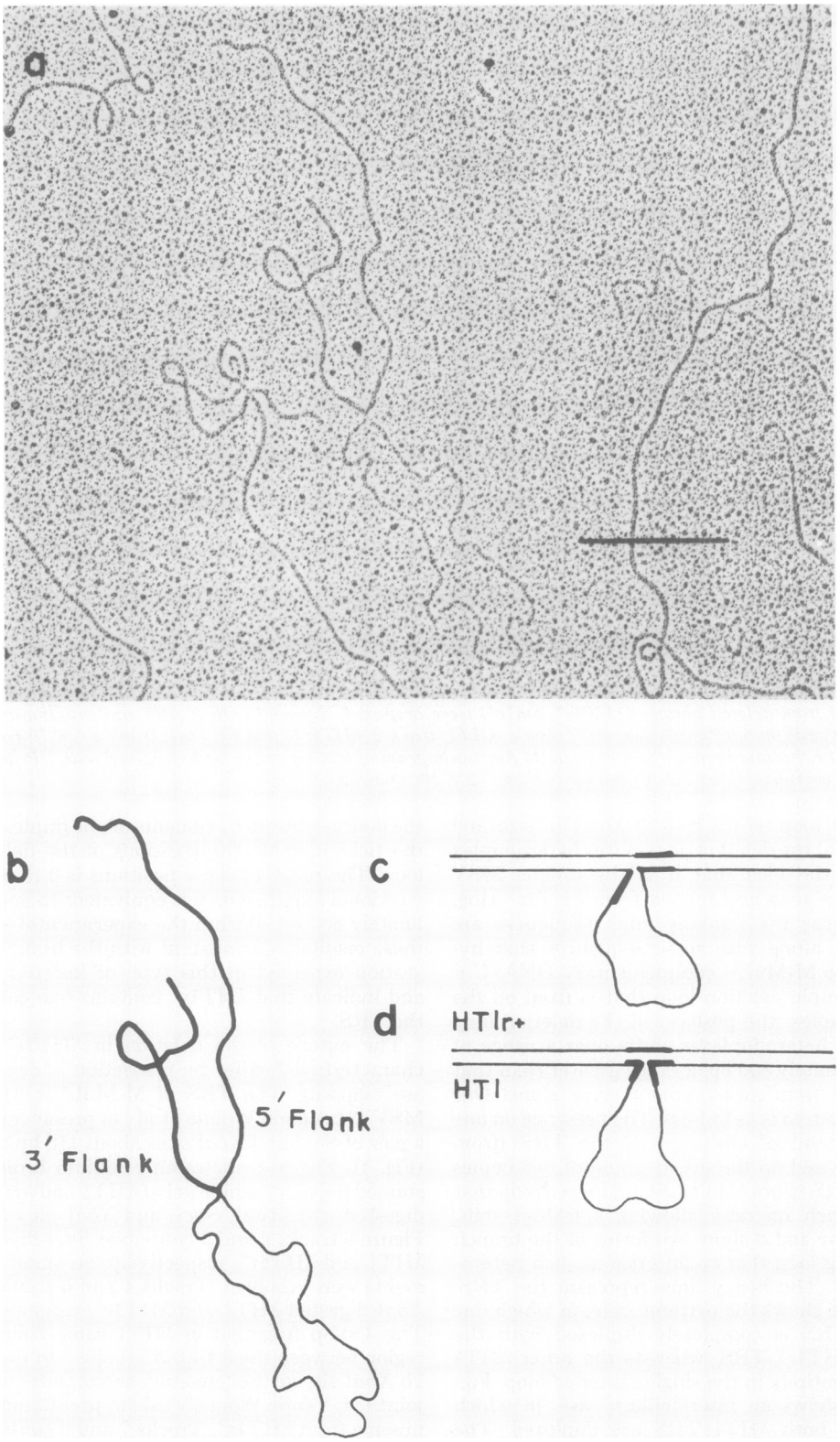

FIG. 4. *Heteroduplex of λHT1 and λHT1r⁺ DNAs. DNAs from λHT1 and λHT1r⁺ were digested with EcoRI before heteroduplexes were formed to eliminate the participation of λ vector arms. (a) Heteroduplex. Bar = 1 μm. (b) Tracing of heteroduplex in (a). (c and d) Two schematic interpretations of the structure in (a) and (b). The thickened lines represent the TRS.*

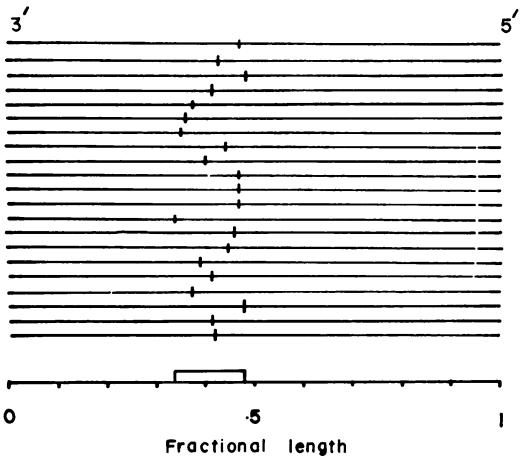

FIG. 5. *Position of the single-stranded loop in λHT1-λHT1r⁺ heteroduplex. The double-stranded segments of 21 heteroduplexes of the inserted DNA fragments of λHT1 and λHT1r⁺ were measured and normalized to the mean length of 6.2 kb. For each linear map the position of the single-stranded loop (unique MSV sequences in λHT1) was determined and is indicated by the tick mark. The range of deletion loop positions shown on the scale line is 0.13 of the total length and is equivalent to approximately 800 bp.*

fragment, (ii) a 421-bp *Sac*I-*Xba*I fragment from the 5′ mink flanking sequence, and (iii) a 118-bp *Xba*I-*Sac*I fragment from the TRS. In λHT1r⁺ (Fig. 6, track 8), the 421-bp flank fragment and the 118-bp TRS fragment were present, but the 450-bp *Xba*I fragment from the unique MSV region was missing. The two *Sac*I sites (one in the flank, one in the TRS) indicate that only one copy of the TRS was present (Fig. 7). Thus, the size of the λHT1r⁺ insert and its restriction map suggest that only the flanking mink sequences and one copy of the TRS were retained. Moreover, blot hybridization and heteroduplex analysis are consistent with this interpretation.

Isolation, cloning, and characterization of an HT1 MSV integration site. A DNA sequence that carries a potential integration site for HT1 MSV was detected in a two-dimensional analysis of *Eco*RI-digested normal mink genomic DNA by using λHT1 as a hybridization probe (Fig. 3) (34). Vande Woude et al. (34; Vande Woude et al., in press) identified this fragment as one carrying the HT1 integration site based on the following findings: (i) it hybridized to the λHT1 probe but not to the λm1 probe; and (ii) its size (5.6 kb) was the sum of the sequences flanking integrated HT1 MSV.

This specific fragment was further enriched by preparative gel electrophoresis and was cloned in λgtWES·λB, as previously described (34; Vande Woude et al., in press). Of 5,000 plaques examined, 3 hybridized with the inserted fragment from λHT1. These were purified, and the DNA was characterized. All hybrids carried an identical 5.6-kb *Eco*RI fragment. The recombinant phage studied most extensively is called λFHT1. Restriction endonuclease mapping of this fragment revealed the same *Sac*I, *Bam*HI, *Xho*I, and *Kpn*I sites as those mapped in the 5′ mink sequences flanking integrated HT1 MSV (data not shown) (Fig. 1).

Direct evidence that λFHT1 carries the HT1 integration site was obtained by using the novel deletion mutant λHT1r⁺. Heteroduplexes were formed between *Eco*RI-digested λFHT1 and λHT1r⁺ DNAs (Fig. 8). The two fragments were homologous except for a small deletion bubble (620 ± 90 bases) which was 3.46 ± 0.14 kb from one end and 2.14 ± 0.06 kb from the other end. From its size and position, this deletion loop appears to represent the entire single copy of the MSV terminal repeat in λHT1r⁺. From this result we conclude that the 5.6-kb sequence is coextensive with the flanking mink sequences in λHT1r⁺, which are in turn coextensive with the flanking mink sequences in λHT1. Thus, the 5.6-kb fragment carries the sequence used by HT1 MSV for integration. At this level of resolution, it appears that the provirus can integrate by simple insertion. It also appears that the normal integration site does not contain any extensive sequence homology with the MSV TRS.

DISCUSSION

High-frequency spontaneous deletions of λMSV hybrids. During lytic growth, spontaneous deletions of λ occur normally at a frequency of about 1 in 10^6 to 10^7 phage (37). The endpoints of these deletions can be more or less random unless the phage integration-excision system is active (15). In this system, deletions are more frequent, and one end of the deletion is often at the phage attachment site. The λgtWES vector used in this work is missing the integration-excision system, as well as the phage-encoded general recombination functions (*red*), so no λ-promoted site-specific deletions are expected in recombinant phages (17). As we show here, one class of deletion mutation occurred frequently in stocks of λMSV hybrids. This result was not unexpected because restriction endonuclease mapping of λm1 and λHT1 indicated that the integrated m1 and HT1 MSV proviruses were bracketed by TRS. Homologous repeating units of this type are known to be substrates for the general recombination systems of *E. coli* (3, 22). Because the TRS were direct and not inverted, homologous recombi-

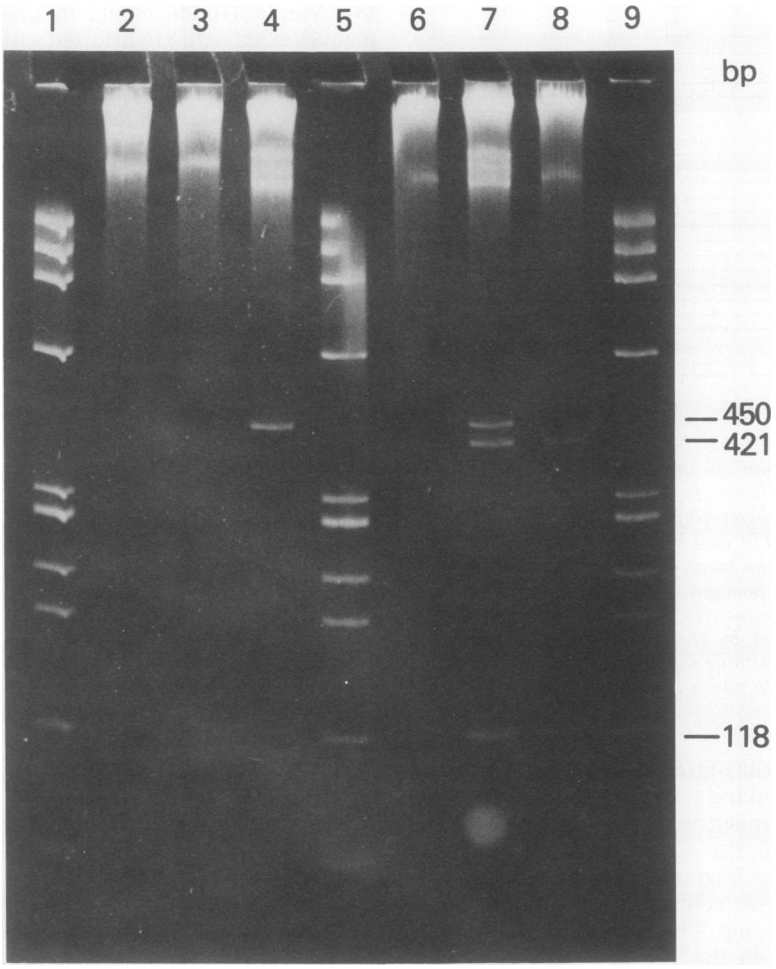

FIG. 6. *Restriction endonuclease analysis of λHT1 and λHT1r⁺. λHT1 DNA (tracks 2, 4, and 7) and λHT1r⁺ DNA (tracks 3, 6, and 8) were digested with SacI (tracks 2 and 3), XbaI (tracks 4 and 6), and XbaI plus SacI (tracks 7 and 8). Tracks 1, 5, and 9 contained HaeIII-digested φX replicative form DNA as a size standard. Digested DNAs were fractionated on a 10% acrylamide gel and stained with ethidium bromide. Because of the low level of DNA, the 118-bp XbaI-SacI fragment in λHT1r⁺ (track 8) is difficult to detect in photographic reproductions.*

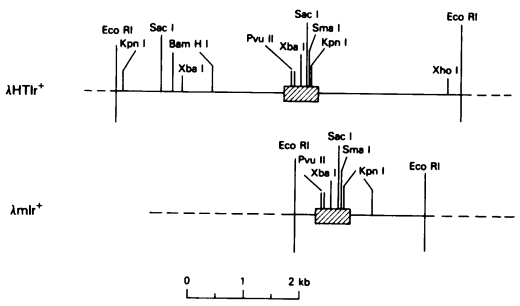

FIG. 7. *Physical maps of λM1r⁺ and λHT1r⁺. See legend to Fig. 1.*

nation between them in an intramolecular event or in an intermolecular event by unequal crossing over was predicted to generate phages that lost the DNA between the repeats. (Duplications can also be generated by unequal crossing over, but the phage DNA carrying such duplications would be too large to be packaged into viable phage particles.) Indeed, our characterization of several such λMSV deletions gave credence to this idea. However, preliminary experiments indicate that these same deletions of λMSV hybrids may occur in recombination-deficient *recA*⁻ hosts (unpublished data). Although the mechanism of excision is not known, these dele-

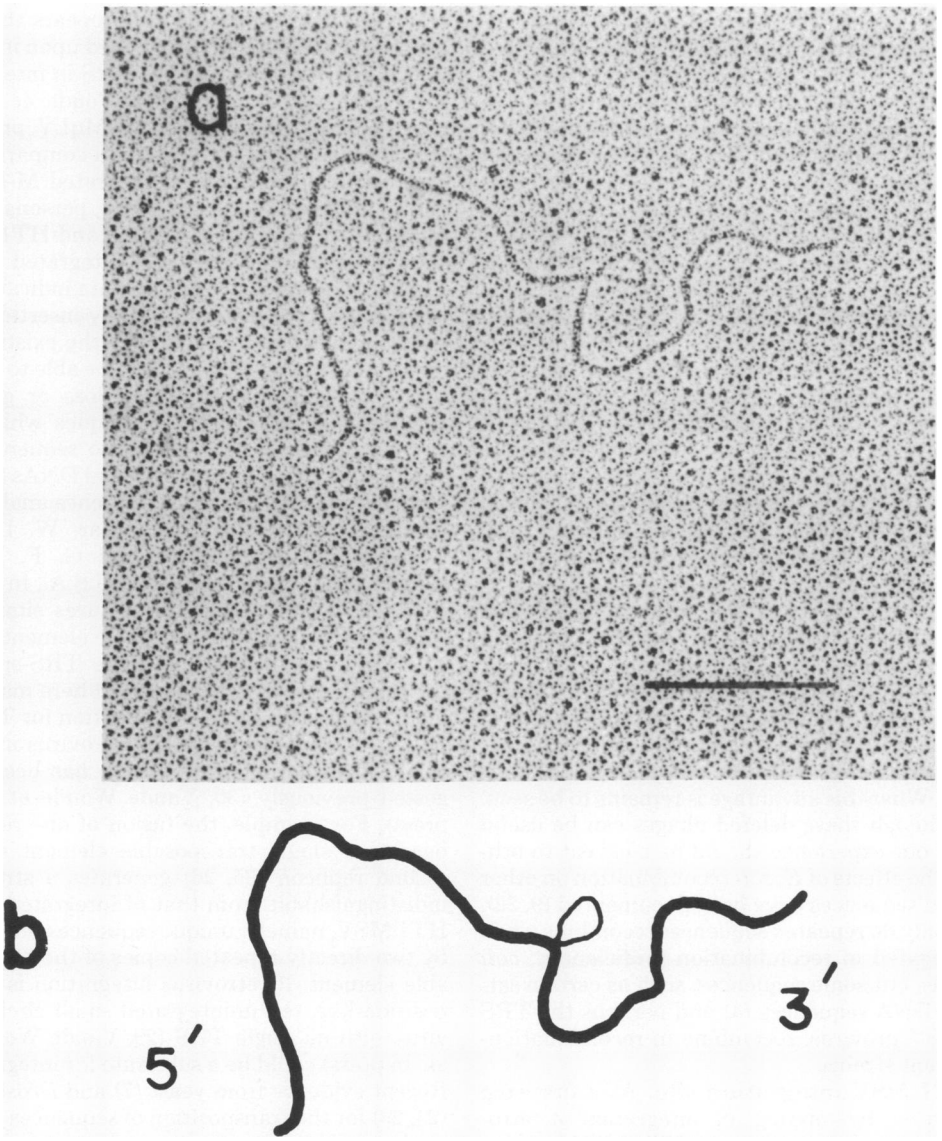

FIG. 8. *Heteroduplex of λHT1r⁺ and λFHT1. DNAs from λHT1 and λFHT1 were digested with EcoRI to eliminate participation of λ vector arms. (a) Heteroduplex. Bar = 1 μm. (b) Schematic representation. The average lengths of individual features of six molecules were: 5′ flank, 3.41 ± 0.14 kb; single-stranded loop, 0.62 ± 0.09 kb; 3′ flank, 2.16 ± 0.06 kb.*

tions strongly resemble the "*cam* cut-out" deletion mediated by the insertion element IS*1* (19, 25). In this reaction, the chloramphenicol resistance transposon Tn*9*, which contains directly repeated copies of the 800-bp IS*1* sequence at both termini, is specifically deleted from λ, leaving one copy of IS*1* behind. The *cam* cut-out deletion is independent of *E. coli recA* and λ *red* recombination systems. If the deletion of MSV proviral sequences occurs via the same mechanism, it indicates that the procaryotic transposition system can recognize eucaryotic sequences and suggests a role for MSV TRS in *E. coli* similar to that of the transposable elements. It remains to be determined whether TRS can function similarly in eucaryotes.

The high proportion of λHT1r⁺ in λHT1 stocks was striking. In some lysates, as much as 50% of the phage was λHT1r⁺. This is in contrast to similar stocks of λm1, in which little or no

deleted phage could be detected. When an additional unrelated *Eco*RI fragment was inserted into λm1 (λm1ff), lysates of the resulting phage contained λm1r$^+$ in detectable quantities, although never at the levels observed for λHT1. The reason for these differences involves at least the following three factors: the size of DNA capable of being encapsidated by λgtWES, the selective growth advantage of one λgtWES hybrid over another during mixed growth, and the time of occurrence of the deletion event. The smallest *Eco*RI fragment that can be inserted in λgtWES and produce a viable phage is between 2 and 3 kb (Enquist, unpublished data; A. Skalka, personal communication; J. Davison, personal communication). This accounts for our inability to find λm1r$^+$ in λm1 stocks, because the loss of about 5 kb from λm1 makes the phage DNA too short to be packaged in a stable form. When the 43-kb λm1 genome was enlarged to 50 kb (λm1ff), the 45-kb λm1r$^+$ deletion was readily detected.

The seemingly higher frequency of deletion formation by λHT1 compared with λm1ff may reflect some selective advantage of λHT1r$^+$ over λHT1 that λm1r$^+$ does not enjoy over λm1ff. Because lysates are made by multiple rounds of growth and not by single cycle infection, any small advantage would be amplified in the final yield. What this advantage is remains to be seen.

Although these deleted phages can be useful tools, our experience should be a caveat to others. The effects of *E. coli* recombination on other cloned sequences have been documented (9, 22). Not only do repeated sequences recombine when propagated in recombination proficient *E. coli* strains, but some sequences, such as certain satellite DNA sequences (4) and perhaps the TRS of MSV provirus, recombine in recombination-deficient strains.

HT1 MSV integration site. As a first step toward understanding the integration of retroviruses, we characterized an HT1 MSV integration site by heteroduplex analysis and restriction enzyme cleavage. At this level of resolution, we found no major rearrangement of host sequences caused by integration of the provirus. Since the heteroduplex between the λHT1r$^+$ and λFHT1 inserts shows a simple deletion loop, the integration site does not appear to have any sequences that are lost when the provirus integrates. The size of the deletion loop (620 ± 90 bases) is equal to the size of the TRS, and this suggests that there is no host sequence homologous to the TRS or that if there is, it is quite small. This is consistent with the results of Varmus et al. (35), who could not detect repeat-like sequences in avian hosts by using 3′ and 5′ sequences of avian retroviruses as probes. It also appears that the proviral sequence is not perturbed upon integration. Restriction maps of the TRS in integrated MSV proviruses (34; Vande Woude et al., in press) and in unintegrated M-MuLV provirus (11) are identical. Furthermore, a comparison of the restriction maps of unintegrated M-MuLV (11) and MSV 124 (1, 8; D. Dina, personal communication) with integrated m1 and HT1 MSV shows the unintegrated and integrated proviruses to be coextensive. These data indicate that the provirus integrates by simply inserting into the host genome, thus splitting the existing sequence. However, we would not be able to detect subtle rearrangements or the loss or gain of small sequences by the techniques which we employed. Our current efforts to sequence the TRS and flanking host and normal DNAs should detect any such subtlety. A sequence analysis of the TRS of m1 MSV (R. Dhar, W. L. McClements, L. W. Enquist, and G. F. Vande Woude, Proc. Natl. Acad. Sci. U.S.A., in press) revealed several structural features similar to those of procaryotic transposable elements (5, 6, 12, 13). These features and the TRS-specific, high-frequency deletion reported here may provide evidence for an IS-like function for TRS in *E. coli*. An analogy between retrovirus integration and bacterial transposition has been suggested previously (30; Vande Woude et al., in press). For example, the fusion of one replicon bearing a single transposable element with a second replicon (25, 26) generates a structure indistinguishable from that of integrated m1 or HT1 MSV, namely, unique sequences bracketed by two directly repeated copies of the transposable element. If retrovirus integration is transposition-like, the unintegrated small circle provirus with its single TRS (29; Vande Woude et al., in press) could be a substrate for integration. Recent evidence from yeast (7) and *Drosophila* (23, 29) for the transposition of sequences bracketed by direct repeats suggests that transposition (and integration) mediated by directly repeated sequences may be a common phenomenon in eucaryotes. Thus, models proposed for transposition in procaryotes (5, 25) may provide insight into the mechanism of retrovirus integration. The cloned MSV proviruses and the normal integration site should help elucidate this mechanism by providing substrates for sequence analysis and in vitro integration studies.

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